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NOTES ON THE BIOLOGY AND A DESCRIPTION OF THE EGG, THIRD INSTAR LARVA AND PUPA OF *NEOBISNIUS SOBRINUS* (COLEOPTERA: STAPHYLINIDAE)

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ABSTRACT

The egg, third instar larva and pupa of *Neobisnius sobrinus* (Erichson) are described and illustrations of structural features are provided. Both adults and larvae are cryptic and hygrophilous, living in moist organic debris along streams and ponds. The species appears to have a polyvoltine life cycle. Larval-rearing techniques are described and data presented on growth of immatures. This first detailed description of any *Neobisnius* larva indicates that the genus differs from related genera in having three stemmata on each side of the head, a mandible with seriations on the inner margin, and sharp cuticular spines on the inner surface of the first segment of the urogomphus.

† † †

Neobisnius sobrinus (Erichson) is a cryptic, hygrophilous staphylinid beetle distributed from Newfoundland through North and Central America to Northern South America (Frank, 1981). It is the only entirely castaneous species of *Neobisnius* found in Nebraska and is easily separated from other staphylinids by its unique habitus (Fig. 28). Little information has been published on *Neobisnius* immatures. Topp (1978) included *Neobisnius* in a key to genera of European staphylinid larvae, with one figure of *N. cerrutii* Gridelli larvae (nasale). Newton (1990) included *Neobisnius* (grouped with *Philonthus* and *Belonuchus*) in a key to genera of North America staphylinid larvae based on Topp (1978). This paper describes the egg, larva and pupa of a *Neobisnius* species for the first time and includes notes from the field and the laboratory on the biology and life history of *N. sobrinus*.

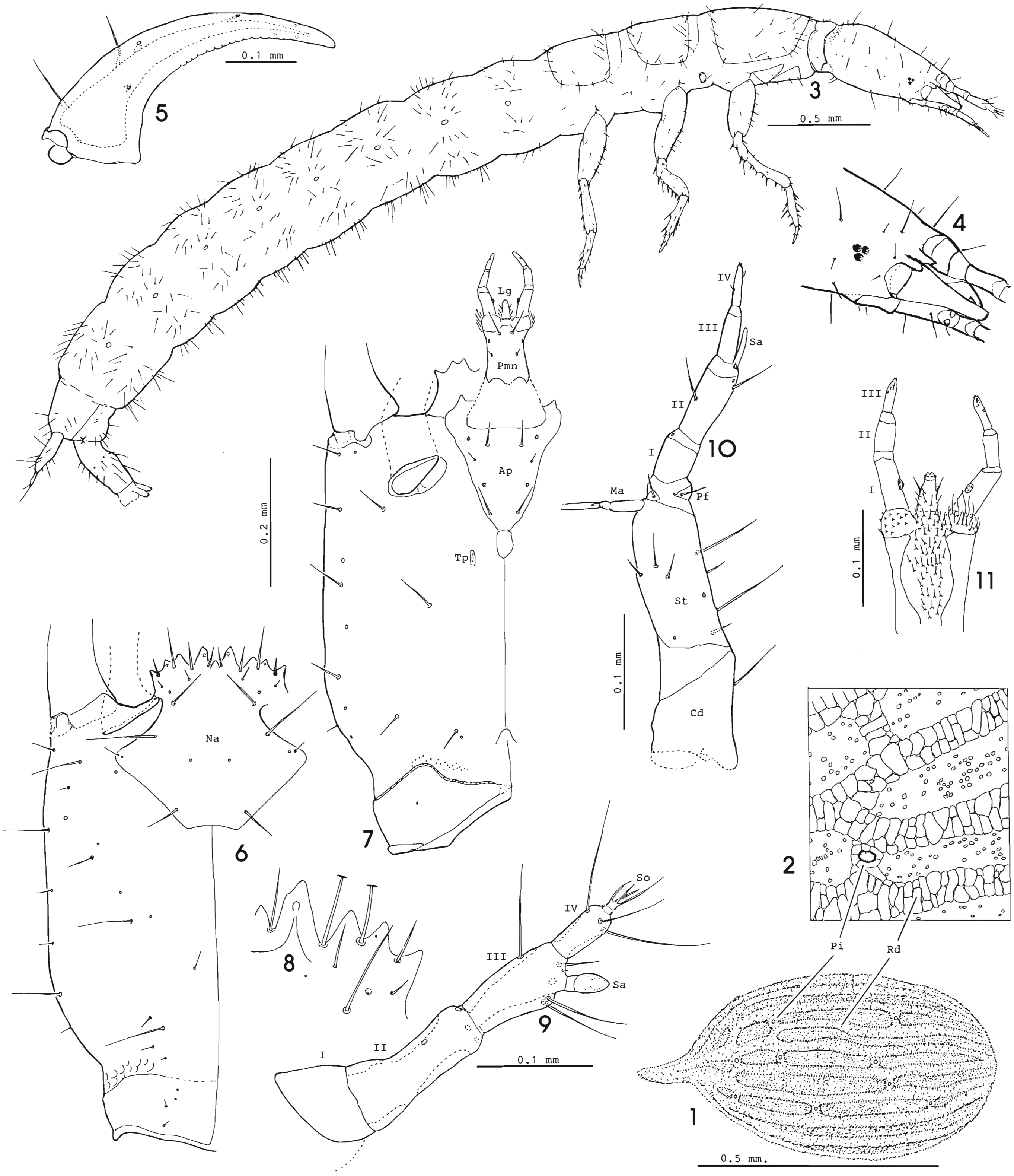
MATERIALS AND METHODS

The study site was next to a lowland creek meandering through a pasture 4 mi (6.5 km) W and 4 mi S of Schuyler, Colfax County, Nebraska. Adults were collected at the water's edge and in adjacent moist, low-lying areas using three methods: (1) sifting organic debris, (2) trapping in unbaited pitfall traps, and (3) forcing the beetles up into the open by pulling on plant stems or splashing water over an area of ground. Most of the collected adults were placed in terraria stocked with soil and plants from the collecting area and were observed to eat Coleoptera larvae (Oxytelinae gen. spp.), and Diptera larvae, pupae, and adults brought in with the material used to construct the terraria. *Drosophila* larvae were later added as additional food. Occasionally, collected females were kept in standard containers and checked after 12 hours for oviposition (Table 1).

Standard containers for rearing were made from 45 × 30 mm plastic cups with snap-on plastic lids. A 5-mm layer of plaster of Paris was poured into the bottom of each cup and, after hardening, covered with a 0.5-mm layer of sandy loam. Holes were punched in each lid with a No. 1 insect pin and all containers were kept in the dark in larger, covered plastic boxes. The soil layer was occasionally stirred to control mold hyphae. In

Table 1. Summary of data on *N. sobrinus* females ovipositing within 12 hours of collection.

Date Collected	No. of Females checked	No. Ovi-positing	Percentage
4 April 1992	1	1	100
14 April 1992	3	3	100
28 April 1992	8	8	100
4 June 1992	1	1	100
4 July 1992	2	2	100
5 August 1992	3	3	100
4 Sept. 1992	3	1	33
12 Sept. 1992	26	1	4



Figures **1–11**, *Neobisnius sobrinus*. **1–2**, egg. **1**, lateral aspect. **2**, surface of chorion at 400x. **3–11**, larval instar III. **3**, habitus, lateral aspect. **4**, right stemmata cluster. **5**, left mandible, dorsal aspect. **6**, head, dorsal aspect. **7**, head, ventral aspect. **8**, nasale, right half, dorsal aspect. **9**, left antenna, dorsal aspect. **10**, left maxilla, ventral aspect. **11**, labium, dorsal aspect with pubescence on left half removed. **Ap**, apotome; **Cd**, cardo; **Lg**, ligula; **Ma**, mala; **Na**, nasale; **Pf**, palpifer; **Pi**, aeropyle on connection between ridges; **Pmn**, prementum; **Rd**, ridge; **Sa**, sensory appendage; **So**, solenidium; **St**, stipes; **Tp**, tentorial pit.

containers with larvae, sandy loam was gradually added up to 4.0 mm depth to allow each larva to form a pupal chamber of compressed soil. Laboratory temperature was held at 25°C±1 except where noted, and all containers were checked once a day.

Specimens examined: Twelve eggs, 8 chorions from hatched eggs, 29 third instar larvae, 6 third instar exuviae, and 6 pupae (all reared).

Measurements and drawings were made using a binocular dissecting microscope equipped with camera lucida. Eggs and chorions from hatched eggs were stained with Lignin Pink stain. Freshly killed, freeze-dried, uncleared alcoholic, and KOH-treated specimens of the eggs, larvae, and pupae were examined. Exuviae and examples of all immature stages were recorded, collected and preserved as required. Voucher specimens were placed at the University of Nebraska State Museum, Lincoln, and at the Field Museum of Natural History, Chicago. Identification of adults was confirmed by J. H. Frank, Entomology and Nematology Department, University of Florida, Gainesville.

IMMATURE STAGES OF *NEOBISNIUS SOBRINUS*

The description is largely modeled on recent descriptions of larvae of other staphylinid subfamilies by Watrous (1981), Ashe and Watrous (1984), and Frania (1986).

Egg:

Ridges on egg (Figs. 1, 2) opaque white with area between ridges translucent grayish white. "Tail" on end composed of converging ridges, ridges forming slight pucker at opposite end. Connections between ridges slightly lower than ridges, most with aeropyle in middle rising slightly higher than ridges. Egg flaccid when laid, average length 0.65 mm (0.75 mm including tail), height 0.25 mm, width 0.35 mm (7 eggs measured, S.D. = ±0.01). By third day, length same, height and width both 0.40 mm (same 7 eggs as above measured, S.D. = ±0.01).

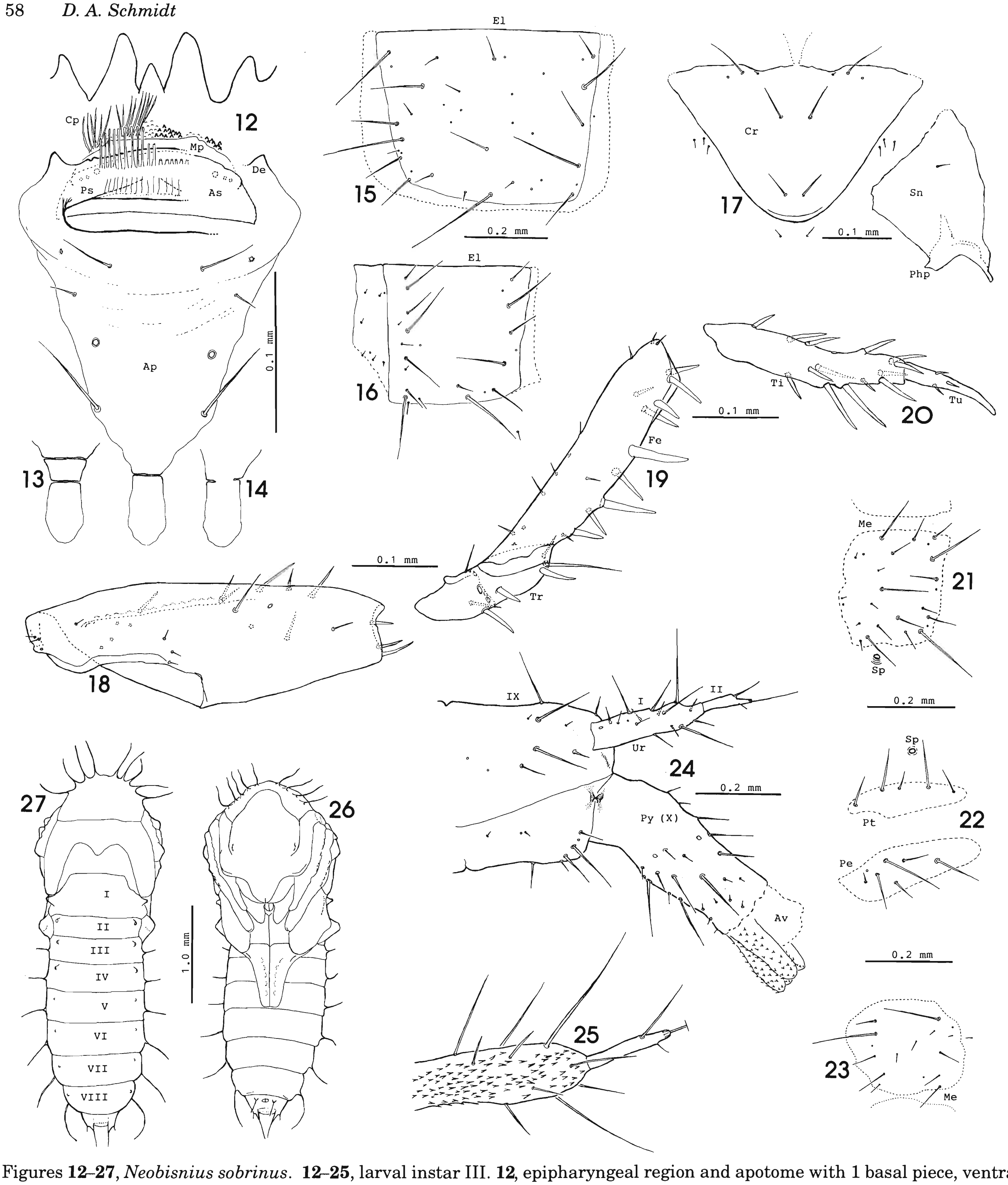
Larva (III instar):

Body length 4.5–5.5 mm (Fig. 3). **Color** of live larva: head light reddish brown, color fading to almost transparent from head to metanotum. Legs light yellowish brown. Setae light yellowish brown to dark brown. Remainder of body white to transparent. **Vestiture** of simple setae only except posterior seta on each paratergite VIII frayed.

Head: (Figs. 3–14), longer than wide, side margins parallel, 3 stemmata present each side (Fig. 4), no lenses apparent. Antenna with 4 segments (Fig. 9); segment I without setae or pores; segment II with 1 pore dorsally, 3 pores apically; segment III with 3 setae medially, 1 macro and 1 micro seta, and 1 pore ventroapically; sensory appendage transparent with sclerotized band at base; segment IV with 3 setae and 4 solenidia apically, 1 solenidium longer than other 3. Mandible (Fig. 5) with seriations on inner margin, 2 setae on outer margin, 2 pores on dorsal surface. Nasale (Fig. 8), anterior margin with 9 teeth divided into 3 distinct clusters with 3 teeth each cluster. Epipharyngeal region (Fig. 12) with numerous cuticular processes on ventral side of nasale. Membranous patch (**Mp**) between cuticular processes and sclerite (**As**). Sclerite (**As**) forming dorsal half of mouth, smooth with 3 pair of denticles. Sclerite (**Ps**) forming ventral half of mouth, with one row long narrow cuticular processes between angular denticles. Apotome convex at apical end, flattening towards base, basal tip generally separate piece, occasionally of 2 pieces (Figs. 12, 13), rarely connected with apotome (Fig. 14). Maxilla (Fig. 10), cardo with single seta on ventral outer margin, stipes with 4 setae and 1 pore on outer margin, 3 setae and 1 pore ventral inner margin, dorsobasal three-fourths membranous and glabrous. Mala with 2 long and 1 short sensilla at apex, 4 small sensilla on dorsal side of apex. Palpifer continuous in dorsal aspect with arms extending around each side in ventral aspect, 1 seta on end of each arm. Palp with 4 segments; segment I with 1 pore ventrally on apical inner margin; segment II with 1 seta and 1 pore on apical outer margin, 1 seta on inner margin; segment III with 1 digitiform sensory appendage basolaterally; segment IV with 1 seta on inner margin, 1 seta on apical outer margin. Labium (Figs. 7, 11), ventral side of prementum sclerotized with 4 setae, 2 pores, sclerites extending around margins of dorsal side of prementum, remainder of dorsal side membranous, moderately pubescent, ligula sclerotized dorsally and ventrally except for membranous apex, moderately pubescent dorsally. Palps with 3 segments; base of segment I membranous with pubescence on dorsal side, button-like sensory appendage on inner margin; segment II without setae or pores; segment III with 1 pore on outer margin.

Thorax: Pro-, meso-, and metanotum (Figs. 15–16) each with mid-longitudinal ecdysial line. Large spiracle in membrane posteriolaterally of pronotum, small atrophied spiracle in membrane posteriolaterally of mesonotum (Fig 3). Cervicosternum as in figure 17. Prosternal area with 2 sternites, meso- and metasternal areas entirely membranous except for sclerotized catepleura. Coxa as in figure 18. Trochanter (Fig. 19) divided. Femur as in figure 19. Tibia as in figure 20. Protibia without comb. Tarsungulus (Fig. 20) trisetose.

Abdomen: Abdominal terga (Fig. 21) I–VIII divided by membranous area mid-longitudinally, length



Figures **12–27**, *Neobisnius sobrinus*. **12–25**, larval instar III. **12**, epipharyngeal region and apotome with 1 basal piece, ventral aspect with left half of cuticular processes cut off to show pattern and left medial portion of sclerite (Ps) removed to show sclerite (As) beneath it. **13**, with 2 basal pieces. **14**, with basal tip connected with apotome. **15**, left pronotum. **16**, left mesonotum. **17**, cervicosternum and left sternite. **18–20**, right leg of mesothorax, posterior aspect. **18**, coxa. **19**, trochanter and femur. **20**, tibia and tarsungulus. **21**, left abdominal tergite II. **22**, left abdominal paratergite and parasternite III. **23**, left abdominal sternite III. **24**, abdominal segment IX, left urogomphus, and pygopod, lateral aspect. **25**, inner face of right urogomphus. **26–27**, pupa. **26**, ventral aspect. **27**, dorsal aspect. **Ap**, apotome; **As**, sclerite, dorsal half of mouth; **Av**, anal vesicle; **Cp**, cuticular processes; **Cr**, cervicosternum; **De**, denticle; **El**, mid-longitudinal ecdysial line; **Fe**, femur; **Me**, membranous area mid-longitudinally; **Mp**, membranous patch; **Pe**, parasternite; **Php**, prehypopleuron; **Ps**, sclerite, ventral half of mouth; **Pt**, paratergite; **Py**, pygopod; **Sn**, sternite; **Sp**, spiracle; **Ti**, tibia; **Tr**, trochanter; **Tu**, tarsungulus; **Ur**, urogomphus.

of tergal plates increasing caudad. Spiracles I–VIII present between tergites and paratergites. One pair of paratergites (Fig. 22) on segments II–VIII, absent on segment I. One pair of parasternites (Fig. 22) present on segments I–VIII. Sternites (Fig. 23) I–VIII divided by membranous area mid-longitudinally, length of sternal plates increasing caudad. Sternum IX entire. Tergum IX entire with two articulated urogomphi (Fig. 24,25) each with 2 segments, segment 1 with sharp, short, cuticular processes on inner face, inner face of segment 2 smooth. Pygopod, segment X (Fig. 24), with anal vesicle entirely membranous, 2 pairs of lobes anteriolaterally of vesicle, 1 pair each side, recurved spines present on lobes.

Pupa: (Figs. 26, 27)

Length 3.5–4.0 mm, width 1.0–1.3 mm, obtect, abdomen not moveable, lightly sclerotized, dirty white to light cream with outer margins and outshoots darker. Anterior margin of prothorax bearing 9 setiform projections. Tergosternal sutures present laterally on abdominal segments. One setiform projection present on each outer margin of abdominal segments III–VIII. Spiracles I–IV tuberculate and functional, spiracles V–VIII marked externally but appear atrophied.

Diagnosis:

Characters that distinguish this from related genera are: (1) A ridged egg with a "tail," (2) larva with simple setae only except for the posterior seta on each paratergite VIII which is frayed, (3) three stemmata on each side of the head, (4) mandible with seriations on inner margin, (5) anterior margin of nasale with nine teeth, (6) maxillary palp four-segmented, (7) labial palp three-segmented, (8) button-like sensory appendage on inner margin of segment I of labial palp, (9) lack of a protibial comb, (10) tarsungulus with three setae, (11) urogomphus as long as apical abdominal segment, (12) sharp cuticular spines on the inner surface of the first segment of the urogomphus; (13) pupa having nine setae-form projections on the anterior margin of the prothorax, and (14) one setae-form projection on each lateral margin of abdominal segments III–VIII. Characters 3, 4, and 12 appear to be unique among the genera of Staphylininae and should be sufficient to separate this from other genera.

In Topp's (1978) key to the subfamilies of the Staphylinidae, the character of four stemmata on each side of the head is given in couplet 28 for the known genera of Staphylininae. This character for the subfamily was also used by Newton's (1990) key based on Topp. The five freshly killed and seven freeze-dried third-instar larvae examined in this study each had three distinct stemmata on each side of the head. In contrast, the alcoholic specimens examined occasionally had fuzzy or partially dissolved stemmata and

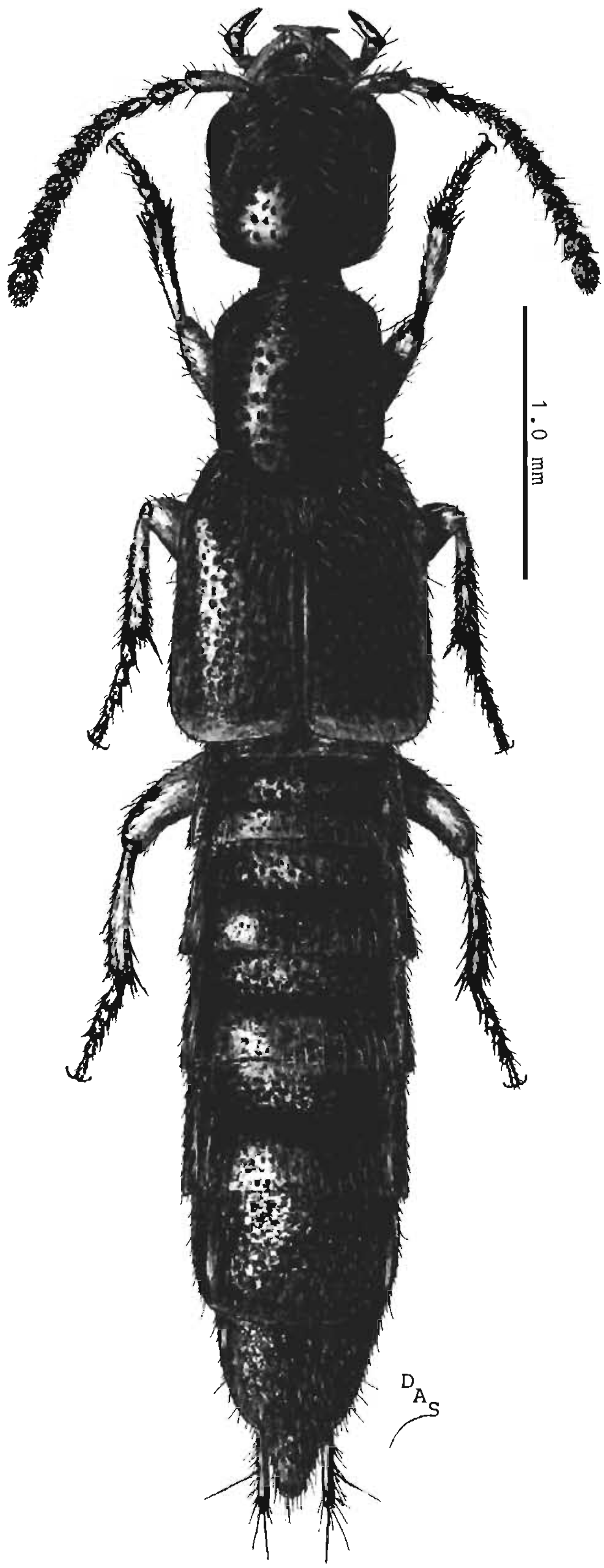


Figure 28. *Neobisnius sobrinus*. Dorsal habitus of adult.

Table 2. Duration of eggs, larval instars, prepupae and pupae of *Neobisnius sobrinus* (*n* = number of observations).

Stage	Duration (days)	$\bar{x} \pm$ S.D. (days)	*n*
	At 25°C±1		
Egg	4.5–5.5	5.0 ± 0.3	17
Instar I	2.5–5.0	3.5 ± 0.8	13
Instar II	2.0–4.0	2.8 ± 0.5	13
Instar III	4.5–9.0	6.8 ± 1.4	8
Prepupa	1.5–5.5	3.1 ± 1.2	7
Total as larva	14.0–17.0	15.5 ± 1.0	7
Pupa	8.5–9.0	8.6 ± 0.4	5
	At 12°C±0.5		
Egg	15.0–17.0	16.0 ± 0.4	16
	At 19°C±0.5		
Total as larva	22.0–24.0	23.5 ± 0.5	7
Pupa	12.5–13.5	13.0 ± 0.3	7

that, along with the absence of visible lenses, might have led to a false stemmata count by Topp. The remaining couplets in Topp's (1978) and Newton's (1990) keys leading to *Neobisnius* agree with this study.

BIOLOGY

Neobisnius sobrinus adults were found in the organic debris along the moist edges of streams and ponds during dry weather but appeared to disperse widely during damp weather. One-hundred forty-two adults were collected between 17 March 1991 and 10 November 1992 with the following distribution: April (6 adults collected); May (15); June (8); July (12); August (35); September (53); October (12); November (1). Third instar larvae were collected in May (1), August (1), and September (1).

In the terraria, eggs were deposited randomly as adults foraged for food. Eggs were sticky only on the end of the small "tail" and were often attached to the substrate by this "tail." Females kept individually in standard containers were found to lay an average of 1.1 eggs a day ($n = 60$, S.D. = ±0.6). Durations of the immature stages are presented in Table 2.

Larvae were aggressive predators and moved with great speed through openings in the soil and debris, even at temperatures ranging down to 18°C. As the temperature lowered further, movement slowed until complete stupor occurred at 5°C. Activity resumed as temperature rose. Pupation occurred in a chamber formed of compressed soil. Full color and hardening of sclerites became complete two to four days after adult emergence.

Five larvae were allowed to reach adult emergence (three females, two males) and were put together in a standard rearing container and maintained at 25°C±1. Eighteen days later the first egg (fertile) was found in the container and an average of 2.8 eggs a day ($n = 7$, S.D. = 0.64) was found in the container for the next seven days (95% of the eggs hatched). The five adults were then slowly brought down to a temperature of 12°C±0.5 over a three day period and held there for two weeks. The females continued to lay eggs but at an average rate of 1.40 eggs a day ($n = 14$, S.D. = 0.44) or half of the rate at 25°C±1. The eggs were held at 12°C±0.5 and 90% hatched at an average of 16.0 days after oviposition ($n = 18$, S.D. = 0.47) compared to 4.5 to 5.0 days average for eggs held at 25°C±1 (Table 2).

DISCUSSION

The life history of *N. sobrinus* in Nebraska appears to be polyvoltine. Adults break winter dormancy and begin ovipositing in April. During the typically wetter months of May and June, a general dispersal pattern is observed with adults being relatively hard to find and collect. Larvae maturing into adults during this time begin to lay eggs after a brief maturation period, allowing the species at least two generations per year. July and August generally bring dryer and hotter conditions which congregate adults in the organic debris along the moist edges of streams and ponds. Adult numbers in these micro-habitats increase steadily over these months, peaking during the first half of September. Sampling indicates that almost all of these probably newly emerged adults are not ovipositioning. Numbers go sharply down as cooler fall weather appears and they completely disappear by November.

Evidence for this life cycle is as follows: (1) The collection of ovipositing females in April, laboratory observations that females can oviposit at 12°C and larvae can mature at 19°C, and the collection of a third instar larva in May would all indicate that new adults would be emerging by the first of June. An 18-day maturation period after adult emergence, as observed in the laboratory, would allow oviposition by the end of June. (2) Collection data for adults and personal observation by the author indicate that the number of adults is at the highest by September which would appear to be too late in the summer for a univoltine species that begins oviposition in April. (3) Females collected in September showed a very low incidence of oviposition. However, that was probably indicative of a large number of newly emerged adults which had not had the proper amount of time to mature. Rearing data on temperature effects on other stages on growth suggests that the cooler temperatures of fall might also retard the adult maturation process, thereby creating a large number of females not ovipositing. Nonovipositing females collected in September, if held at warmer temperatures (25°C±1) in terraria simulating natural conditions and with exposure to natural light, generally began to oviposit within 14 days.

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